Biogenicity of Mn-mineralized Microbes from a Seafloor-Paleohydrothermal Vent System, Vani, Milos, Greece: Relevance to Mn-oxide Biomineralization

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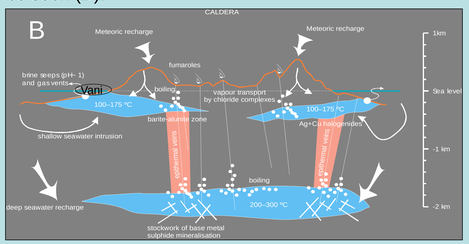
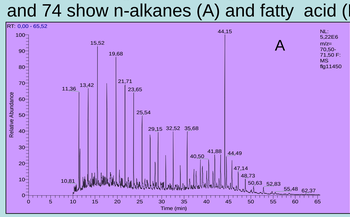
1. The life-supporting geological and geochemical environment

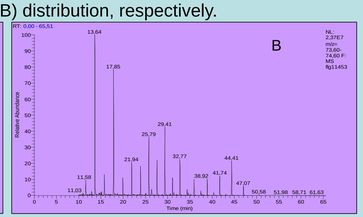
Milos (A), Aegean Sea, is a recently emergent (2 Ma) volcano that documents the transition between the submarine, and terrestrial, volcanic and hydrothermal environments. Here, a shallow geothermal reservoir corresponds to steam-heated zone in epithermal systems, a deep metalliferous geothermal reservoir corresponds to deep, base-metal–rich zone seen in epithermal systems and a network of precious metal epithermal style veins links two reservoirs (B)[1]. Vani represents a rare example of a Quaternary volcanic–hosted hybrid VMS–epithermal-like Mn-oxide deposit formed by a low-temperature (approx. 100ºC) and sunlit/shallow-marine (< 100 m) seafloor paleo-hydrothermal vent system. This comprises (i) mound-style seafloor–exhalative Mn-oxide mineralization in the form of "black" smoker chimneys and chimney rubble, in hydrothermally altered bioturbated volcanic sandstone (C); (ii) volcaniclastic sediment mound in an area of extinct submarine venting (now on land!) with toppled, coalesced and standing (still!) silica–barite–Mn-oxide chimneys near the summit and on the flanks. In addition, trace-fossil of *Ophiomorpha nodosa* can also be seen (D).



3. Biomarkers (molecular fossils) as geochemical indicators of bacterial activity

The neutral lipid, acid and phospholipid fraction of sixteen(16) Mn ore samples from the Vani deposit were analyzed with GC-MS. The detected n-alkanes range from C_{18} to C_{41}. A uniform distribution was observed at all sites, superimposed by increased concentrations of C_{19}–C_{23} alkanes. Major acyclic compounds are fatty acids from C_{15} to C_{36}. The carbon number distribution is similar to that of n-alkanes, with increased concentrations of C_{14}–C_{18}, and maximising at C_{16} and C_{18} fatty acids. Fatty acids show a slight even-over-odd carbon number predominance. The organic compounds also consist of branched C_{18}-C_{23} n-alkanes and C_{15}-C_{18} fatty acids. The presence of straight and branched n-alkanes suggests that the organic matter is thermally altered. C_{14}-C_{20} fatty acids are consistent with a bacterial source and branched fatty acids are generally thought to be diagnostic for bacteria. The mass chromatograms of m/z 71 and 74 show n-alkanes (A) and fatty acid (B) distribution, respectively.

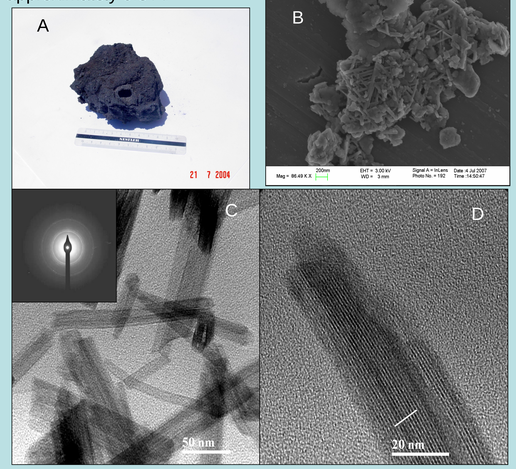




5. Biominerals

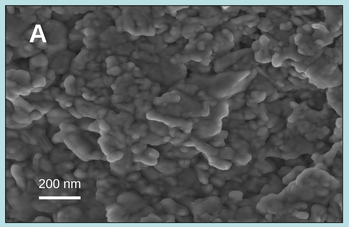
Nanocrystalline todorokite associated with microbial mats formed in seawater

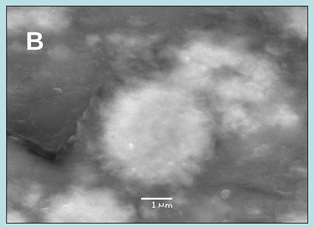
(A) Mn chimney with biogenic todorokite; (B) FEG-SEM image of nanocrystalline todorokite (needles) tightly bound to bacterial sufaces and biofilms representing nascent bacteriogenic precipitates; (C) High–resolution TEM image showing typical nanocrystalline todorokite morphology where nanoparticles are striated elongated platelets. Selected area electron diffraction (SAED) pattern showing diffraction rings at 2.4 Å. EDS analyses show that todorokite holds significant amounts of metals, including Pb, Zn and Ba (D) High-resolution TEM image of todorokite shown in (C) showing lattice fringes at a spacing of approximately 9.8 Å.

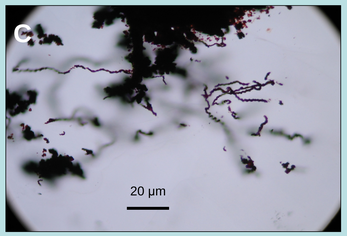


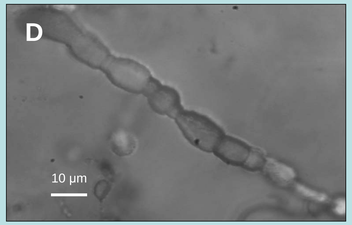
2. Syngenesis of Mn-oxide minerals and microbial fossil bioindicators

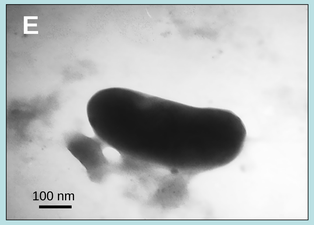
(A) FEG-SEM micrograph of Mn-mineralized biofilm, i.e. extracellular polymeric substances (EPS) and bacterial fossils(budding).
(B) (SEM-BSE) bacterial cells from a fossil colony exhibiting range of metabolic states.
(C) (LM-silica) twisted filamentous bacterial fossils showing coiling and budding.
(D) (LM-silica) cyanobacterium-like septate filament
(E,F) TEM micrographs of (ultramicro) bacteria vibrio cells associated with Mn-oxide nanoparticles

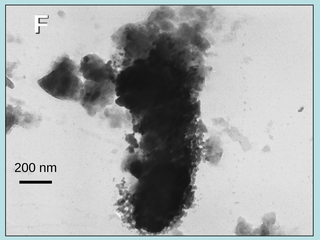








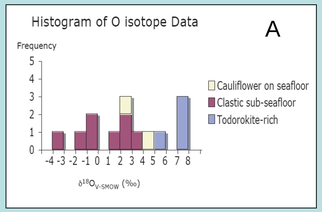




4. Isotope evidence for marine biogenic Mn–oxides

Manganese oxides are formed in sea water via bacterial catalysis of the oxidation of dissolved Mn(II) to Mn(IV)[2]. Preliminary O isotope analysis of a selection of highly purified Milos Mn oxides was undertaken to represent typical groupings. All samples underwent heated pre-fluorination to remove inter-layer water, prior to standard total laser fluorination. $\delta^{18}O$ values range from –4 ‰ to +8‰ (A). The wide range of O isotope data confirm that a complex interplay between oxygen derived from water and dissolved oxygen, and possibly hydrothermal fluids, is involved in the genesis of these Mn oxides, with some data permitting direct microbial involvement. According to the pioneering work of Mandernack et al (1995)[3], microbially mediated single step oxidative precipitation of Mn-oxides display essentially no fractionation, and results in $\delta^{18}O$ similar to the parent water and incorporated molecular O_2(50:50). Inorganic chemical two step oxidation fractionates $\delta^{18}O_{molecular\ oxygen}$ from –13 to –22‰, and $\delta^{18}O_{water}$ between -5 to +1‰ [3]. More elevated $\delta^{18}O$ values ~12‰ (i.e. assuming $\delta^{18}O$: 0 to +2.2‰ for Mediterranean seawater, and +23.5 ‰ for dissolved atmospheric O_2[5]) is more easily understood to involve biological mechanisms involving greater contributions of dissolved O_2, lower values indicating more involvement of O from seawater in this case. Intriguingly, those samples enriched in todorokite also show the most consistently enriched $\delta^{18}O$ values (A) which we tentatively suggest is a very interesting fact in combination with independent evidence for biological origins of todorokite (see inset #5). In addition, it should be noted that $\delta^{18}O$ values of Mn-oxides between 0 and +2.2 ‰ could also be partly "biological" as they may have incorporated maximum dissolved light molecular O_2 produced by marine photosythesizers within the photic zone[4]. In summer months, when photosynthesis is greatest, then a bias towards low values might be seen, whereas when photosynthesis is low, then the usual high dissolved $\delta^{18}O$ value dominates. In such a dynamic environment, where hydrothermal activity impacts on the shallow hydrosphere, it is not surprising that we get a large variation in Mn oxide $\delta^{18}O$, compared to deep sea hydrothermal sites, because we have the possibility of substantial dissolved oxygen contributions from atmospheric, and photosynthetic dissolved, oxygen, in seawater as well as O in hydrothermal fluids.

However, at this preliminary stage, it is not possible to de-convolve all the factors that play a role in arriving at the final measured $\delta^{18}O$ of our Mn oxides, e.g., temperature, fractionation between the oxide and O_2, fractionation between the oxide and H_2O.



6. Conclusions

We present five independent lines of evidence, from the macro- to the nano-scales, that show a close spatial association between metalliferous (Mn±Pb, Zn etc) mineralisation and biological processes, namely:

- The geological environment [Inset #1]
- Bacteriomorphous structures in hydrothermal minerals [Inset #2]
- Biomarkers in manganese ore [Inset #3]
- Oxygen isotope data compatible with microbial involvement in metal precipitation [Inset #4]
- The identification of todorokite—a Mn mineral with demonstrated biological origins [Inset #5]

Individually, these observations do not provide conclusive evidence that microbial activity has played a significant role in mineral precipitation in the Vani seafloor paleohydrothermal vent system. However, when taken collectively, we believe that the data indicate a clear role for biological processes in metal sequestration in the shallow submarine hydrothermal environment.

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